8 Abstract

New insights have been made on the molecular level into the the pathomechanism by which a subgroup of stromal-predominant Wilms tumors with *CTNNB1* and *WT1* mutations emerge. The focal point of this work was the role played by the proto-oncogene CTNNB1 which is stabilized by mutations, thus leading to the activation of the Wnt/β-Catenin-pathway.
Initially, it was known that somatic mutations in the *CTNNB1* gene occur in approximately 15% of Wilms tumors. These mutations are often associated with mutations in the *WT1* gene locus and result in the translocation of the CTNNB1 protein to the cell nucleus. In order to confirm this, various molecular analyses were carried out at the DNA and protein levels. Through *CTNNB1* mutation analysis of total tumor DNA from a large Wilms tumor patient collective and, subsequently, of DNA from microdissected tumor regions of different histological cell types, the molecular basis of *CTNNB1* mutations in Wilms tumors was established. The following results are pertinent to the emergence of tumors:

1.) With a mutation rate of 30%, *CTNNB1* mutations in exon 3 of the gene are a commonly observed phenomenon in the development of Wilms tumors.
2.) In Wilms tumors there exists a 100% invariable association of *WT1* mutations with *CTNNB1* mutations. This cooperation most certainly plays a role in the emergence of tumors with stromal-predominant and triphasic histology but is less relevant in tumors belonging to other histological subgroups.
3.) The genetic heterogeneity of *CTNNB1* mutations proves the molecular multifocality of these tumors and shows that tumor cells with a loss of WT1 function are exposed to strong selective pressure for *CTNNB1* mutations.

Further investigation into the subcellular localization of CTNNB1 and the expression of CTNNB1 and WT1 proteins in Wilms tumors with and without *CTNNB1* and *WT1* mutations yielded new insights into the underlying mechanisms of Wilms tumor emergence. The comparison between blastemal- and epithelial-predominant tumors, which express high levels of WT1, and stromal-predominent tumors, which express little to no WT1 and large amounts of CTNNB1, confirmed the loss of function of WT1 and the stabilization of CTNNB1 in stromal-predominant tumor cells. The stabilization of CTNNB1 leads to continuous cell proliferation and inhibition of the mesenchymal-epithelial transition during kidney development through the activation of the Wnt/β-Catenin pathway. Subsequently, this leads to the deregulation of mesenchymal differentiation which mainly results in the formation of rhabdomyblasts. The translocation of CTNNB1 into the cell nucleus, which activates the Wnt/β-Catenin pathway, was observed in only a few stromal-predominant tumors with *CTNNB1* mutations. This indicates that the nuclear localization on CTNNB1 is not a direct consequence of *CTNNB1* mutations, but rather the result of further genetic, epigenetic or other environmental factors.

For the first time, long term cell cultures of Wilms tumor cells with germline *WT1* and *CTNNB1* mutations could be established for functional studies of tumors from this subgroup. Through this *in vitro* model system, further meaningful insights could be made into the effects of WT1 and a deregulated Wnt/β-Catenin pathway on tumorgenesis. The characterization of tumor cells showed that they represent a very early stage of mesenchymal kidney cell predecessors and possess an active Wnt/β-Catenin pathway. The transcription profile of these tumor cells revealed a strong similarity to mesenchymal stem cells. This confirmed that the emergence of stromal-predominant Wilms tumors is based upon the transformation of a mesenchymal stem cell and that the established cell cultures still possess many characteristics of stem cells. Amongst the tumor specific genes, many are known to play an important role in tumor progression, for example, in proliferation, metastasis, angiogenesis, signal transduction and transcription control. The comparison of the expression profiles of tumors amongst each other identified the genes which were specifically deregulated in one of the three tumors with different *CTNNB1* and/or *WT1* mutations. Besides the many genes which are known to be connected to the Wnt/β-Catenin pathway, several kidney and muscle specific genes, regulators of tumor proliferation, progression and metastasis, as well as genes which are indicators of early developmental anomalies were found. In MD305 and 3082 cells, evidence was found of interactions of the Wnt/β-Catenin pathway with other pathways, for example the RAS and Hh pathways. Through targeted inhibition of CBP dependent CTNNB1/TCF mediated transcription, new putative target genes of the Wnt/β-Catenin pathway in Wilms tumors could be identified. In the promoter sequences of 86% of these potential target genes TCF binding sites could be found. This indicates a direct CTNNB1/TCF mediated regulation of the candidate genes which belong to the functional groups of metabolism, cell cycle, differentiation, organogenesis, as well as tumor emergence and progression. Future investigations must resolve which of these genes are indeed physiological target genes. To this end, the ChIP method was established with which the known Wnt/β-Catenin target genes *Cyclin D1* and *DKK1* were confirmed to be direct CTNNB1/TCF target genes over physical binding of their promoter sequences to CTNNB1.

Finally, evidence was found of a negative regulation of *CTNNB1* through wild type WT1. Through the transfection of wild type *WT1* into HEK293 cells, a down regulation of *CTNNB1* mRNA could be observed.